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Integrating NZVI and carbon substrates in a non-pumping reactive wells array for the remediation of a nitrate contaminated aquifer / Hosseini, Seiyed Mossa; Tosco, TIZIANA ANNA ELISABETTA. - In: JOURNAL OF CONTAMINANT HYDROLOGY. - ISSN 0169-7722. - ELETTRONICO. - 179:(2015), pp. 182-195.
[10.1016/j.jconhyd.2015.06.006]

Availability:

This version is available at: 11583/2624367 since: 2015-11-30T09:20:13Z

Publisher:

Elsevier

Published

DOI:10.1016/j.jconhyd.2015.06.006

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Integrating NZVI and Carbon Substrates in a Non-Pumping Reactive Wells Array for the Remediation of a Nitrate Contaminated Aquifer

Published in

Journal of Contaminant Hydrology

Volume 179, August 2015, Pages 182–195

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Abstract

The work explores the efficacy of a biochemical remediation of a nitrate-contaminated aquifer by a combination of nanoscale zero-valent iron (NZVI) and bacteria supported by carbon substrates. Nitrate removal was first assessed in batch tests, and then in a laboratory bench-scale aquifer model (60 *cm* length × 40 *cm* width × 50 *cm* height), in which a background flow was maintained. Water and natural sandy material of a stratified aquifer were used in the tests to enhance the reliability of the results. An array of non-pumping-reactive wells (NPRWs) filled with NZVI ($d_{50}=50$ *nm*, and SSA= 22.5 m^2/g) mixed with carbon substrates (beech sawdust and maize cobs) was installed in the bench-scale aquifer model to intercept the flow and remove nitrate (NO_3^- Conc.= 105 mg/l). The NPRWs array was preferred to a continuous permeable reactive barrier (PRB) since wells can be drilled at greater depths compared to PRBs. The optimal well diameter, spacing among the NPRWs and number of wells in the bench-scale model were designed based on flow simulations using the semi analytical particle tracking (advection) model, PMPATH. An optimal configuration of four wells, 35 mm diameter, and capture width of 1.8 times the well diameter was obtained for a hydraulic conductivity contrast between reactive materials in the wells and aquifer media ($K_{PM}/K_{aq}=16.5$). To avoid excessive proximity between wells, the system was designed so that the capture of the contaminated water was not complete, and several sequential arrays of wells were preferred. To simulate the performance of the array, the water passed through the bench-scale NPRWs system was re-circulated to the aquifer inlet, and a nitrate degradation below the limit target concentration (10 mg/l) was obtained after 13 days (corresponding to 13 arrays of wells in the field). The results of this study demonstrated that using the NZVI-mixed-carbon substrates in the NPRWs system has a great potential for in-situ nitrate reduction in contaminated groundwater. This NPRWs system can be considered a promising and viable technology in deep aquifers.

Keywords: Nano Zero Valent Iron, Non-Pumping Reactive Wells, Nitrate Removal, PMPATH, Aquifer Remediation, Carbon Substrates.

1. Introduction

Drinking water contaminated by nitrate may cause significant adverse effects on human health, such as methemo-globinemia and blue baby disease in infants, and development of cancer after it reduces to nitrite (Haugen et al. 2003; WHO 2005). Biological denitrification, physical adsorption, and chemical reduction are the three viable approaches for in-situ remediation of nitrate-contaminated aquifers. Biological denitrification is, in principle, a major removal mechanism under natural conditions. However, in many contaminated aquifers the activity of nitrate-reducing bacteria is limited, and has to be enhanced and supported by several engineered interventions. Enhancing bacterial action as the only denitrification mechanism usually requires the injection of dissolved chemical substances (e.g., electron acceptor, electron donor, nutrients) (Schufer and Kinzelbach, 1992). As an alternative, since the 1990s, one of the most successful approaches is the realization of permeable reactive barriers (PRBs) of carbon substrates, in different configurations (continuous trenches, funnel-and-gate or reactive vessel) (Day et al. 1999; Tratnyek et al. 2003; Obiri-Nyarko et al. 2014). The successful use of a wide variety of carbon substrates has been reported in the literature, including limestone (Pearson and McDonnell 1975), bionolle (Schipper and Vojvodić-Vuković 2000), sawdust (Boley et al. 2002; Robertson and Cherry 1995), woodchips (Cameron and Schipper, 2010), rice husk (Shao et al., 2008), leaf compost (Delwiche 1981), maize stalks (Greenan et al. 2006), alfalfa (Benner et al. 1997), cotton (Volokita et al. 1996; Della Rocca et al. 2007a,b). In this case, the removal mechanism is usually a combination of biotic degradation, where the carbon substrate supports the microbial growth, and physical adsorption onto the solid matrix in the barrier. The abovementioned materials have been applied either in classical PRBs or in multi-barriers, which are composed by several reactive and sorbing fillings put in series (Obiri-Nyarko et al. 2014; Van Nooten et al. 2008).

Among abiotic approaches to in-situ denitrification, Zero Valent Iron (ZVI) was successfully applied for nitrate removal in the form of millimetric particles in PRBs, both as the only filling material (Cheng et al. 1997; Westerhoff and James 2003), and mixed with carbon substrates, to promote a concurrent chemical reduction of nitrate for an integrated bio-chemical denitrification (e.g., Gillham and O'Hanneisin 1994; Nurmi and Tratnyek 2008; Lien and Wilkin 2005; Della Rocca et al. 2007a). Despite the efficacy of ZVI and carbon substrates as individual materials in PRBs for nitrate removal, the application of individual materials (either ZVI or carbon substrate)

as filler for PRBs may present some disadvantages. ZVI alone is effective, but can result in the production of relevant quantities of ammonium and H_2 bubbles, with a negative impact on the barrier permeability. Moreover, it is sensitive to a progressive decreased reactivity of Fe^0 toward contaminants, due to the formation of mineral precipitates onto the ZVI surface (Della Rocca et al. 2007a; Cheng et al. 1997; Su and Puls 2004). On the other hand, the use of carbon substrates in PRBs may negatively affect the porosity and the hydraulic performance of the barriers in the medium- and long-term as a result of the accumulation of biomass or biofilm and the production of gas bubbles, with consequent diversion of groundwater flow around the barrier (Taylor and Jaffe, 1990; Phillips et al., 2000; Gu et al., 2002). The combined use of a carbon substrate and millimetric ZVI in PRBs for in situ bio-chemical denitrification has been considered in more recent years, and proved effective in improving denitrification rates via autotrophic (AD) and heterotrophic (HD) denitrification processes simultaneously (Della Rocca et al., 2007a,b), overcoming most drawbacks associated to the individual biological or chemical denitrification processes (Till et al., 1998; Huang et al., 1998; Dejournett and Alvarez, 2000; Shin and Cha, 2008). In this sense, the presence of microorganisms can significantly enhance the performance of the reactive barrier by biotic degradation of the contaminants, by consuming H_2 gas bubbles produced by iron corrosion in groundwater (Weathers et al., 1997; Novak et al., 1998; Li et al., 2011). Moreover, the integrated ZVI-bacteria system does not require intensive maintenance and constant supply of organic substrates, produces less biomass sludge, allows a rapid and complete process at nearly neutral pH, and results in N_2 as a final product, rather than the abiotically produced NH_4^+ in high concentration (Huang et al., 1998; Dejournett and Alvarez, 2000; Shin and Cha, 2008).

In this work the efficiency of two carbon substrates (beech sawdust and maize cobs) mixed with nanoscale zero-valent iron particles is evaluated for nitrate removal in a bench-scale laboratory model which simulates a natural, stratified aquifer heavily contaminated by nitrates from diffuse sources (Shahrekord aquifer, Iran). The two carbon substrates were selected based on their high suitability to support bacterial denitrification, as evidenced in a previous work by Warneke et al. (2011). Contrary to previous works described in the literature, which report the use of millimetric ZVI as filling material in the PRBs, in this work nanoscale zerovalent iron (NZVI) is proposed for use in combination with the carbon substrates. Compared to millimetric or micrometric iron particles (MZVI), NZVI has a much larger specific surface area, which results in a faster

degradation of contaminants (Tratnyek and Johnson, 2006; Huang et al., 2013; Tosco et al., 2014; Karn et al., 2009), but also in an increased corrosion and reduced lifetime, which makes sometimes MZVI preferable for its higher longevity (Velimirovic et al., 2014; Luna et al., 2015). Hazards of NZVI handling during the transportation, handling, and injection of the NZVI are higher compared to MZVI (Renn and Rocco, 2006), and the impact of eventual surface modification (e.g. by noble metals) to enhance NZVI reactivity and stability may increase toxicity (Phenrat et al., 2009). The cost per unit mass of iron is favorable to micrometric and millimetric particles (eg. in 2014 in the USA \$30-40/lb for NZVI, \$1-5/lb for MZVI, \$0.25-0.75/lb for millimetric ZVI) (NanoRem 2014), but if cost is re-scaled over surface area ($\$/\text{m}^2$) it is approximately the same for the three materials. As a consequence, the preference for NZVI rather than MZVI or millimetric iron is not straightforward, and implies several site-specific considerations on costs, hazards, and operational feasibility. In this study NZVI was preferred to MZVI and millimetric iron for coupling with the carbon substrate for its higher reactivity, due to the urgent need of a fast and effective reduction of nitrate in the contaminated aquifer, which is the primary drinking water source of the area and urge to be remediated in a short period.

The second challenge posed by the remediation of the Shahrekord aquifer is related to the depth of the contamination (down to 70 m). PRBs are typically limited to depths lower than approximately 20 m (USEPA 1998), and consequently are not viable for this application. On the contrary, arrays of non-pumped reactive wells (ANPRWs) have been studied as an appropriate alternative for greater depths (Wilson and Mackay 1997). A typical ANPRWs consists of a series of rows of closely-spaced un-pumped wells filled by fully-penetrating cartridges containing the reactive materials, which perform as a discontinued PRB (Wilson et al., 1997). A comprehensive explanation of scheme, design, and performance modeling of the ANPRWs, and the use of the ANPRWs for amendment delivery, has been well documented by Freethey et al. (2002). Beside the higher depth of treatment, the ANPRWs allow an easier regeneration of the reactive filling compared to PRBs (which is particularly important in this application, due to the relatively short lifetime of NZVI) and removal of the reactive material when the remediation is complete. Moreover, this approach, compared to injection of particle and substrates directly into the subsoil may be considered 'precautionary' by some regulators and industries concerning the possible side effects of releasing nanoparticles into groundwater (e.g. Royal Society 2005, Iranian Ministry of Energy, 2008) by concentrating the nanoparticle into a container.

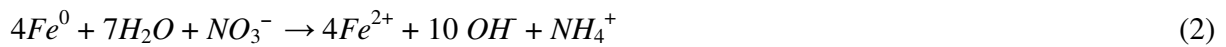
2. Mechanisms of combined autotrophic-heterotrophic denitrification

The combination of bacteria-mediated autotrophic and heterotrophic denitrification in the presence of Fe^0 is not merely a sum of parallel mechanisms, but results in a synergic, complex sequence of concurrent reactions, as previously elucidated into details by Della Rocca et al. (2007a,b) and Liu et al. (2014). Autotrophs use carbon dioxide (CO_2) as a carbon source and hydrogen (H_2) or reduced sulfur compounds as electron donors (Till et al., 1998; Haugen et al., 2002). Heterotrophic denitrification (HD) requires an organic carbon rich environment, which can be achieved by supplying either soluble (sucrose, methanol, ethanol, etc.) or insoluble carbon sources. (eg. vegetable oil, natural organic substrates such as liquorice, giant reed, wheat straw and cotton, bio-polymer and methane) (Rocca et al., 2007).

The main mechanisms of the denitrification process in the presence of NZVI and bacteria are illustrated in Figure 1 according to the general conceptual core-shell structure model. The main processes occurring at the NZVI surface include (i) iron corrosion, (ii) hydrogen production via cathodic depolarization, and (iii) abiotic reduction of nitrate. When Fe^0 is immersed in anoxic water, dissolved hydrogen is produced via cathodic depolarization (Dejournett and Alvarez, 2000):



In parallel, abiotic reduction of nitrate to ammonia by NZVI takes place beyond the tolerance range of denitrifiers (Till et al., 1998; Li et al., 2006):



Even though this process is known to be quite slow at nearly neutral pH, resulting in insignificant ammonia production and increasing groundwater pH (Gayle et al., 1989; Shin and Cha, 2008). The aqueous nitrate ($NO_3^-_{\text{aq}}$) is transferred to the boundary layer of the Fe^0 particles, and then adsorbed as $NO_3^-_{\text{ad}}$. $NO_3^-_{\text{ad}}$ is then diffused along the boundary layer and is chemically reduced to other forms as NH_3_{ad} , $NO_2^-_{\text{ad}}$, and $NH_4^+_{\text{ad}}$. The products in the boundary layer can be desorbed and diffused from the surface into the solution in ionic form, or leave the solution in the gas form. At nearly neutral pH, with the passing of time non-crystal $FeOOH$ is formed on the

particle surface as the main oxidation product of Fe^0 in the presence of denitrifying bacteria. This layer could inhibit further electron transfer from the Fe^0 core to the surface, and consequently decrease the rate of nitrate reduction directly by ZVI (Phillips et al., 2000; Huang and Zhang, 2004). As the pH increases due to OH^- production in reaction (2), the layer of FeOOH on the iron particles can adsorb OH^- in the solution, becoming negatively charged, and consequently preventing to adsorb nitrate on the iron surface. Nevertheless, the cathodic depolarization process (1) is not depleted, since neutral water can still adsorb to the iron surface due to its electrical properties, and thus H_2 can be continuously generated (Li et al., 2011).

Simultaneously, in such an anaerobic environment enriched by cathodic hydrogen H_2 and insoluble carbon substrates, both autotrophic (AD) and heterotrophic (HD) denitrification contribute to nitrate reduction (Gu et al., 2002). These bacteria use nitrate as a terminal electron acceptor in their respiration process in the absence of oxygen through the sequence reactions conducted by different enzymes (Till et al., 1998):



During this process the end products of denitrification are carbon dioxide (CO_2) and nitrogen gas ($\text{N}_{2\text{g}}$) with the formation of a number of nitrogen intermediates: $\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$ (Shin and Cha, 2008). Thus, biochemical reduction of nitrate can enhance both the rate and extent of transformation and yield a more innocuous and favorable end product distribution (Dejournett and Alvarez, 2000).

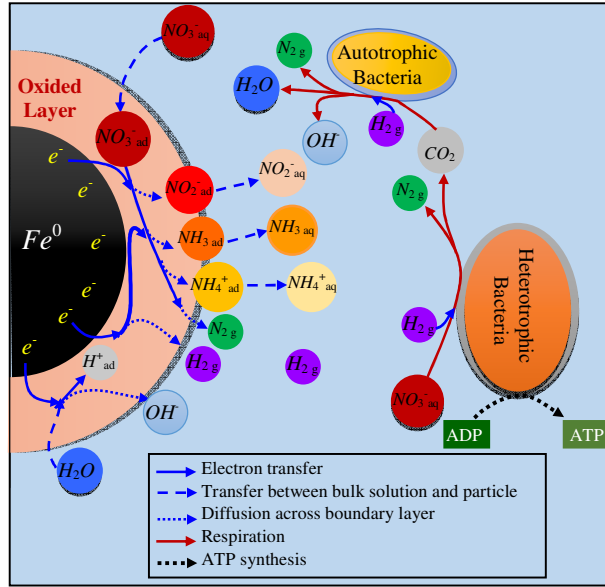


Figure 1: Conceptual model of NO_3^- reduction by NZVI particles in presence of denitrifier bacteria.

3. Site Description

This study is the preliminary part of a full scale remediation project of the Shahrekord contaminated aquifer by Regional Water Organization of Chahar-Mahal and Bakhtiari province. This unconfined aquifer is located in a semi-arid region in the West of Iran, and has an area of 550 km^2 , specific yield=0.03-0.08, transmissivity= $200\text{-}1100 \text{ m}^2/\text{d}$, hydraulic conductivity= $2\text{-}20 \text{ m/d}$, porosity=0.20-0.33, depth to groundwater=25 m (Figure 2), and aquifer bottom (bedrock) at 100 meters or deeper below the ground level. Precipitation is limited, with an annual average of 320 mm. The lack of a permanent river in the plain and the recent rapid population growth led to a progressive decrease of the groundwater recharge and to the overexploitation of the aquifer, which is the only available water resource in this area for several activities. In addition, intensive use of fertilizers by agricultural sectors (which is the dominant land use in this region), leakage from wastewater network, septic tanks and county sewage penetrating wells, and undesired releases wastewater from a treatment plant (Figure 2) (Chahar-Mahal and Bakhtiari Regional Water Company, 2008) resulted in the accumulation of nitrate in the groundwater, which has exceed the permissible concentration level ($\sim 10 \text{ mg/L}$ of N according to Iranian Health Ministry Regulation).

The spatio-temporal variation of nitrate concentration in the groundwater was assessed by water sampling from 10 pumping wells (W1 to W10, as shown in Figure 2) around the installation site

in three sampling campaigns in 2012. The summary statistics of the nitrate concentrations observed in the samples are reported in Table 1. The results in Table 1 indicated that the nitrate concentration in the aquifer has a high variability during the sampling period (standard deviation=42.33 *mg/l*). The maximum concentration (105 *mg/l*- NO_3^-) was observed in the last sampling campaign in well W5, which supplies the tap water of Taqanak town in the outlet plume of groundwater. As a consequence, the natural groundwater collected at the well W5 during the last sampling campaign was used in the laboratory experiments.

The NPRWs system was chosen due to the significant depth of the contaminant plume (36-68 m) and operational and cost aspects. Due to the abundance, spreading, and type (both point and diffuse) of contamination sources, it is nearly impossible to design a NPRWs close to each source. The location of the NPRWs installation was proposed at a location where the aquifer is narrow, the geological properties are more favorable, and there is an accumulation of nitrate in the contaminated plume (Figure 2). In this location, the aquifer is surrounded by rocks and formed in a previous river bed excavated in the bed rock.

Table 1: Summary results of nitrate concentration in groundwater sampling of pumping wells.

Well ID	W1	W2	W3	W4	W5	W6	W7	W8	W9 ^a	W10
Mean (<i>mg/l</i>)	23.1	25.3	20.1	55.0	56.0	22.8	30.4	22.8	31.3	36.0
Standard Deviation (<i>mg/l</i>)	2.40	4.70	0.45	12.90	35.92	3.65	1.40	12.80	42.33	3.06
Groundwater Depth (m)	44	36	58	45	68	53	45	62	44	52

^a Well W9 is located right downstream the outlet of the wastewater plant.

4. Materials and Methods

4.1. Characterization of Reduced Agents

The NZVI particles (NANOFE STAR, NANOIRON, Czech Republic) have a Fe^0 content of 90%, are stabilized by a thin layer of iron oxide, and have an average diameter of 50 *nm*, specific surface area of 20-25 *m*²/*g* (see also <http://nanoiron.cz>). The selected carbon substrates were beech (*Fagus Orientalis*) sawdust (Nitrogen=1.87%, Carbon=38.26%, Hydrogen=6.03%, and Sulphur=1.05%) and maize cobs (Nitrogen=1.12%, Carbon=40.81%, Hydrogen=5.45%, and Sulphur=0) with size of 5±2 *mm*. The chemical composition of the substrates was analyzed by gas chromatography (GC, HayeSep Q 60/80, Shimadzu Co., Japan, ASTM-D3612 Method B) equipped with a thermal conductivity detector (TCD) and two columns.

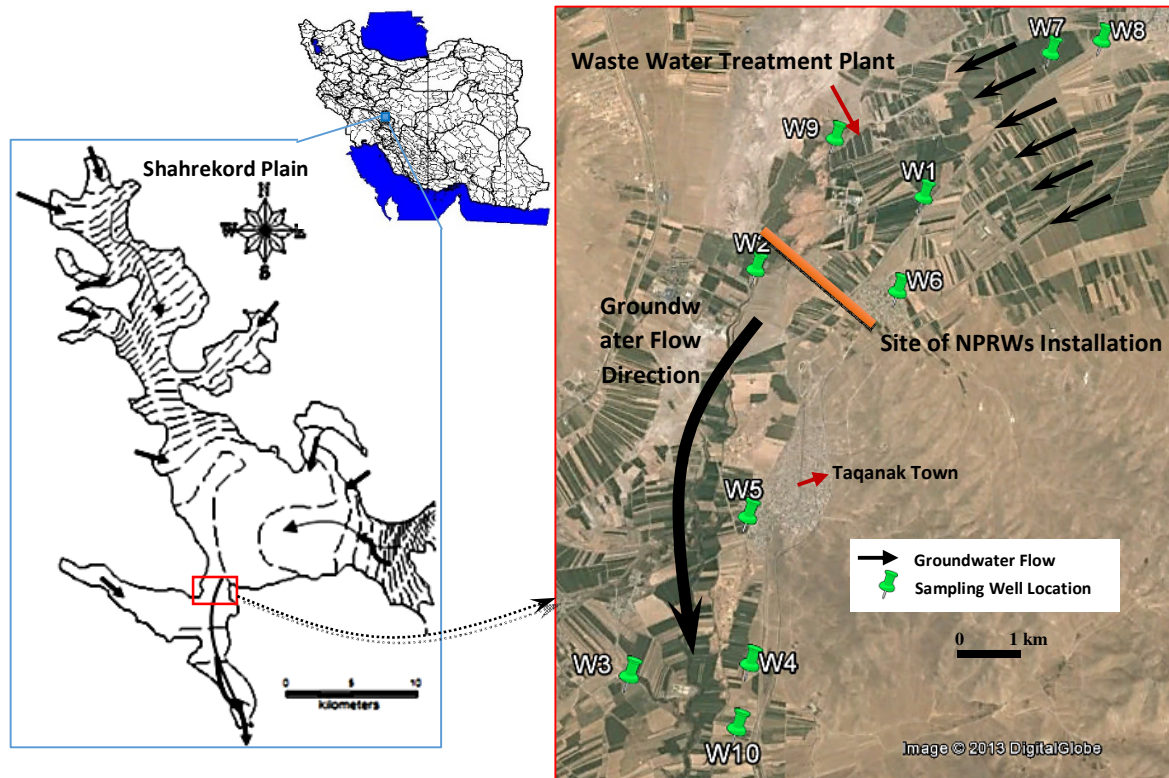


Figure 2: Location of pumping wells for sampling and location of the NPRWs installation in Shahrekord Aquifer.

4.2. Batch Experiments

Batch experiments were performed in bottles (200 ml) filled by six different combinations of the reactive materials (named A to F). Natural water of Sharekord's aquifer (at well W5) with NO_3^- concentration of 105 mg/l was used in all tests. The bottles were kept under ambient conditions ($25 \pm 2^\circ\text{C}$) and were run in duplicate. The Nitrate reductant agents in A and F are carbon substrates and NZVI, respectively, whereas in the others (B to E) mixtures of all reactive materials were used in different ratios. Table 2 indicates the amount of substances for each test.

Table 2: Concentration of carbon substrates and NZVI used in the six treatments.

Substances	Treatment					
	A	B	C	D	E	F
Maize Cobs (g/l)	80	80	80	80	60	0
Beech Sawdust (g/l)	40	40	40	40	60	0
NZVI (g/l)	0	2	4	8	4	4

Water was sampled from the bottles every day using a 2 ml syringe through an opening on the top of each bottles, simultaneously. Then the samples were filtered by a 0.2 μm filter paper just before determination of NO_3^- and NH_4^+ using a UV–Vis spectrophotometer (SPECORD 250-222P205) at wavelength of 500 and 250 nm, respectively, and electrical conductivity (EC) measurement. After each sampling, the space produced by taking 2ml sample on the top of solution was filled by Ar gas. The bottles were shaken slowly to ensure that all the nitrate would have access to the available reductant agents. The experiment was continued until the concentration of nitrate in the bottles declined to an acceptable value according to the drinking water standards (i.e. 10 mg/l).

4.3. Bench-scale Aquifer Design

To evaluate the NO_3^- reduction under flow conditions, a bench-scale laboratory setup mimicking the ANPRWs in the Shahrekord aquifer was built according to the core logs extracted from wells near the remediation site. The aquifer in the area of the remediation site consists of three stratified layers, including clay (top layer with 8-10 m thickness), clayey sand (middle layer with 20-25 m thickness), and clayey gravel (bottom layer with 32-35 m thickness). The thickness of aquifer and the depth to groundwater in this site are 60-70 m and 10 m, respectively. The bench-scale laboratory setup of the aquifer was fabricated in Plexiglas in dimensions of 60 cm (length) \times 40 cm (width) \times 50 cm (height), as shown in Figure 3. The model was filled with three layers of sediment with different particle size and composition which mimicked the field stratigraphy. The materials used to fill the model are clayey soil collected at the site, fine sand ($d_{50}=0.85\text{ mm}$) and gravel ($d_{50}=3.5\pm0.5\text{ mm}$) from Iranian Silica Sand MFG Company.

For the top layer (with thickness of 10 cm), the clayey soil collected at the site was used directly, with hydraulic conductivity (K) equal to 0.004 m/d, and porosity (n) equal to 0.40, according to the laboratory tests. The middle layer (with thickness of 20 cm) consists of sandy clay soil with hydraulic conductivity equal to 1.75 m/d, and porosity equal to 0.30. In this layer, the site clay and fine sand ($d_{50}=0.85\text{ mm}$, $n=0.22$, and $K=3.0\text{ m/d}$) were mixed in the ratio of 45% and 55% w/w. The bottom layer of model aquifer was gravel-clay soil (30 cm thickness) with mixing of clay with fine gravel in ratio of 25% and 75 % w/w, respectively.

The average hydraulic conductivity (\bar{K}), pore water velocity (\bar{V}), and hydraulic gradient (i) of the aquifer model resulted, respectively, in 2.10 m/d, 0.52 m/d, and 0.02. For easier data

collection, a thin layer of fine gravel was put on the top of the aquifer model. The flow velocity in the bench scale aquifer model was selected similar to the one observed in the natural aquifer. The system properties and the applied head difference resulted in residence time approximately equal to 1 day.

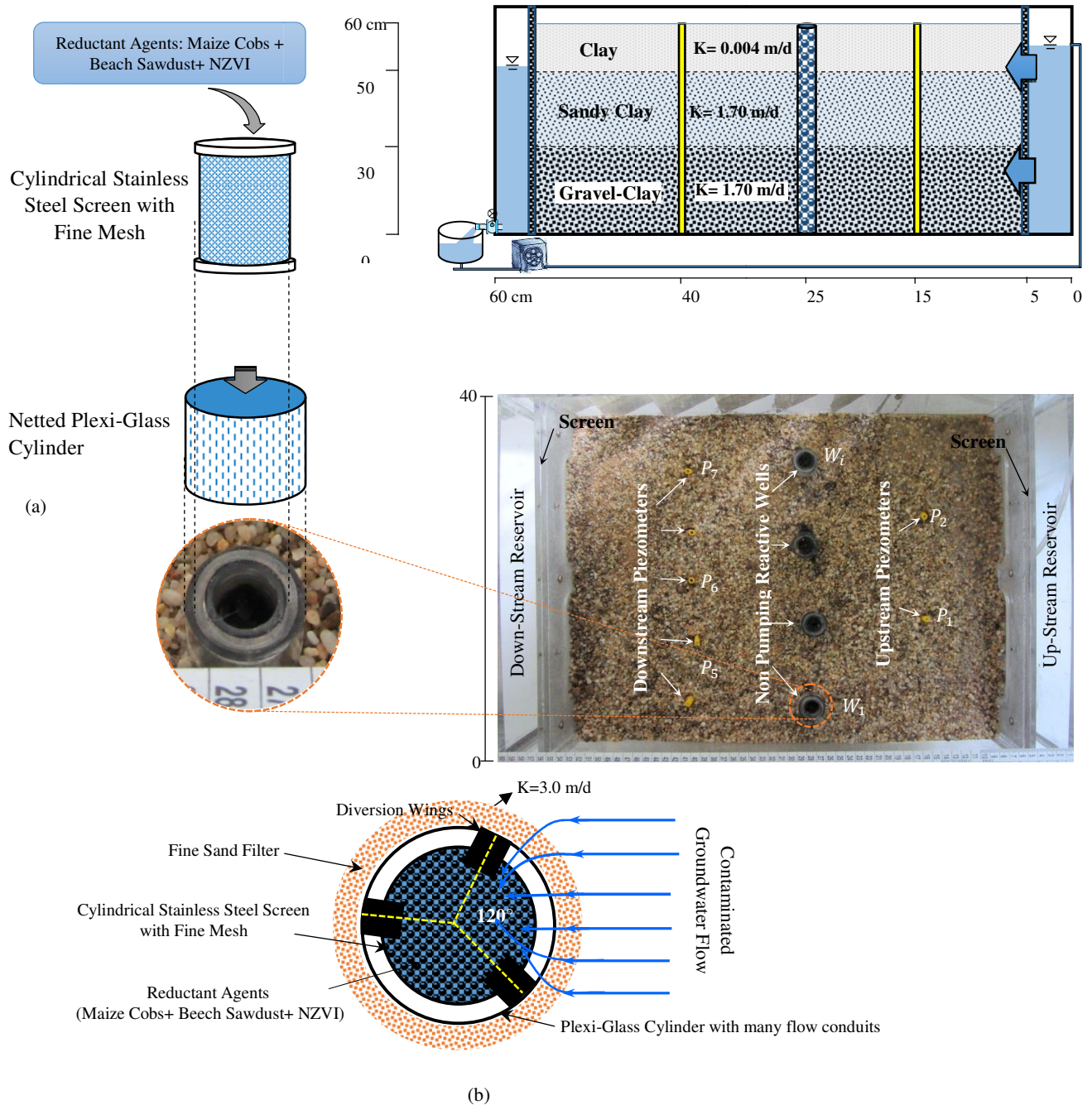


Figure 3: Schematic of Aquifer Laboratory setup (a) and Schematic plan view of a well in NPRWs system (b).

An array of NPRWs (W_1, W_2, \dots, W_N) was emplaced perpendicular to the groundwater flow direction at a distance of 25 cm from the entrance of the model (see Figure 3). Two sets of piezometers were placed upstream (P_1 and P_2) downstream (P_3 to P_7) the NPRWs array, respectively at distance of 15 cm and 40 cm from the model inlet. The final values of the design parameters, i.e. number of wells (N), distance between wells (L_W), and wells diameter (D_W), for the NPRW array in the bench-scale model were determined by numerical simulations. The particle tracking model PMPATH (Pollock, 1994; Chiang and Kinzelbach, 1998) was applied to simulate the groundwater flow in the proximity to the NPRWs system, and to obtain the optimum values of N_W , L_W and D_W in field conditions.

Each well in NPRWs system is composed by three basic parts (Figures 3a-b) an external stiff Plexi-glass cylinder with high capacity flow conduits throughout its length, a cylindrical stainless steel cartridge with fine mesh that contains the reactive materials, and diverging wings to direct the contaminant plume into the reactive cartridge. Three diverging wings with an orientation angle of 120° among them were placed outside the steel cylinder to guide the flow through the well. A thin layer (5 mm) of sand ($d_{50}=0.8$ mm, $K=3.0$ m/d) was used as filter around each well to prevent the soil particles entering into the well screen.

The natural groundwater of the aquifer with background nitrate concentration (C_0) of 105 mg/l and ionic strength (I) of 50 mM was pumped using a peristaltic pump (B-V Series, Etatron D.S., Italy) into the upstream reservoir. Before introducing the cylindrical cartridges containing the reactive material into the wells, 10 pore volumes of the natural groundwater were pumped to the aquifer in order to obtain a uniform distribution of the contaminated water in all parts of the porous medium. A constant hydraulic gradient was ensured by tuning the water level in both upstream and downstream reservoirs. The treated effluent water from the downstream reservoir was circulated and pumped to the upstream reservoir, to simulate parallel, subsequent NPRWs arrays in the aquifer. The recirculation of the contaminated water was continued until the concentration of nitrate in the effluent water had been reduced to the acceptable level.

4.4 Numerical model

The flow in the bench-scale aquifer was simulated using MODFLOW in three dimensions (McDonald and Harbaugh, 1988), by incorporating a three-layered block-centered finite-difference grid of 120 rows and 180 columns with uniform size of 3.5 mm wide. The

hydrodynamic properties of the layers were imposed equal to those reported in the section 4.3. Zero-gradient boundary conditions were applied at the upstream and downstream end of the bench-scale aquifer model, whereas no-flow boundary conditions were kept on the other sides (impervious walls). All simulations were run varying the parameters N_W (1, 2, 3, ..., 6), and D_W (10, 20, 30, 35, 40, 50 mm) in a steady state long-term period (36 simulations). The diameter of the wells (D_W) used in the simulations was selected according to available ones for Plexiglas cylinders. The distance between wells (L_W) did not require independent adjustment since it depends on the values of N_W and D_W , being the total width of the capture front fixed. A semi-analytical particle-tracking tool (PMPATH) was used to track forward 200 particles from the upstream boundaries to delineate the capture zone of wells, using the flow field solved by MODFLOW as an input.

5. Results and Discussions

5.1. Culture of Denitrification Bacteria

The abundance of denitrifying populations in the contaminated water in different treatments (A to F) was assessed by counting the cell number (CN) using the traditional plate count method on an agar background in Petri dish ([Garcia-Armesto et al., 1993](#)). The number of denitrifier bacteria did not grow significantly when the NZVI (sample F, 19.4% increase) or the carbon substrate (bottle A, 37% increase) were the only reactive agent. Conversely, a dramatic increase (437%) of the denitrifier bacteria community was observed in bottle D (mixture of 20g Maize cobs, 10g beech sawdust, and 2.0g NZVI). In addition, for a specific mass of carbon substrates, it was observed that increasing the NZVI concentration resulted in a more relevant growth of the denitrifier bacteria community (treatments from B to C to D). This can be attributed to the availability of the produced cathodic hydrogen (eq. 1) as an energy source for the autotrophic growth of pure cultures of methanogenic, homoacetogenic, and sulfate-reducing bacteria ([Till et al., 1998](#)).

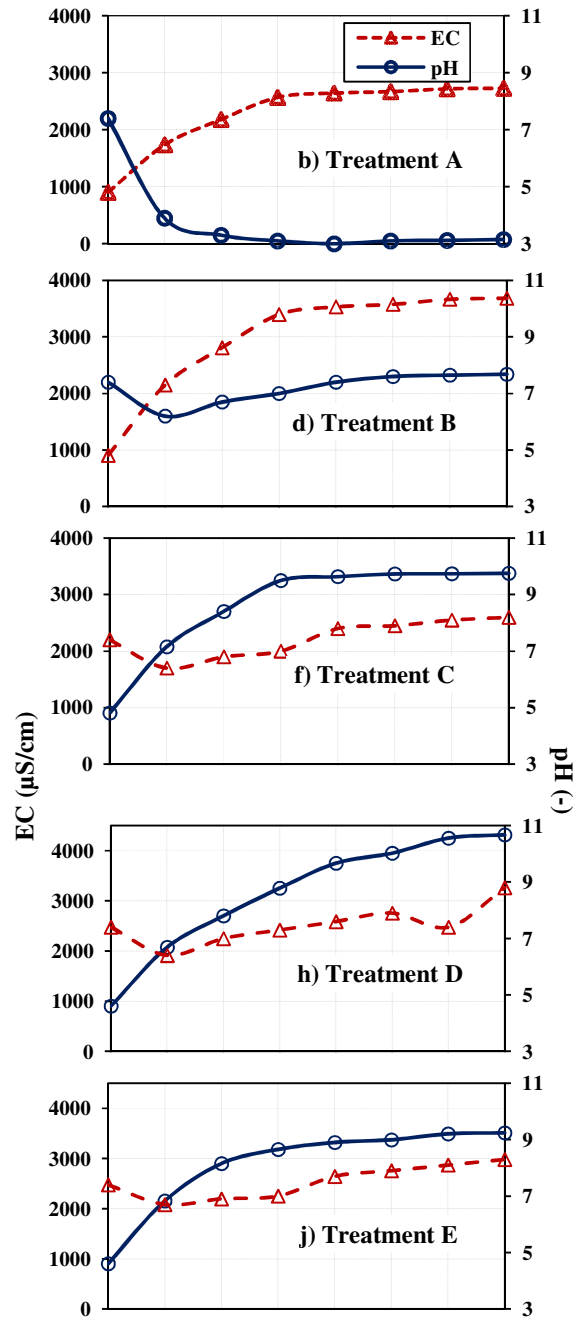
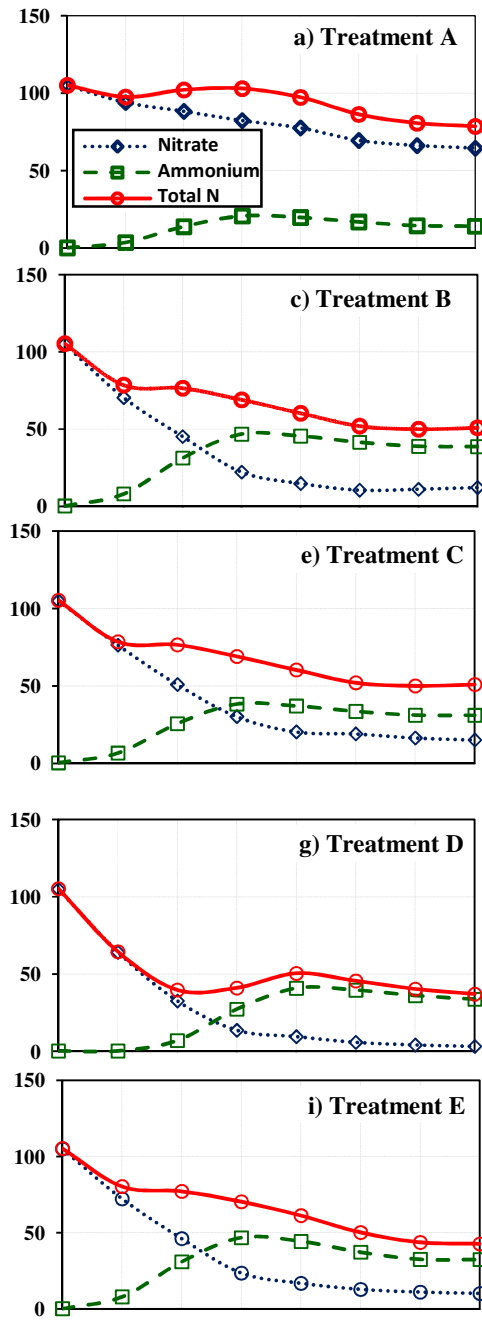
5.2 Batch Denitrification Experiments

The concentration of nitrate, ammonium, total nitrogen (TN), pH, and electrical conductivity (EC) was measured in six batch denitrification tests (Figure 4). The concentration of intermediated products such as ammonia or nitrite was negligible in the solutions during the

experiments. The results indicate that nitrate concentration for treatments containing NZVI (B to F) decreased exponentially, whereas the nitrate reduction rate for treatment A (without NZVI) followed a linear trend. Treatment D, including 30 g of carbon substrates and 2.0 g NZVI (i.e. the highest concentration of both materials), showed the best performance in nitrate reduction (removing 87% of C_0 in 3 days). In this regards, the reciprocal effect of NZVI and denitrifier bacteria provided favorable conditions for nitrate reduction through a combined bio-chemical process. Increasing the NZVI dosage enhanced both nitrate reduction and H_2 production, and this cathodic hydrogen further promoted the denitrifying populations. The enhanced activity of these bacteria increased the carbon dioxide concentration and consequently the pH decreased, promoting the dissolution of the oxidized layers on the iron surface and the reaction progress.

For all curves except the A treatment (i.e. no NZVI), a two-step trend in the degradation can be observed: a first, steep slope (up to 3 days for treatment D, up to 4 days for treatment B to F) followed by a more gradual decrease in the second part of the test. Treatment F, which contained 1.0 g NZVI, exhibited a reduction of 66% after 3 days, while treatment A, containing 30 g carbon substrates with no iron, exhibited only a reduction of 38% after 7 days. It can be perceived that adding the NZVI to the solution had a strong effect on the nitrate reduction (treatments B to E compared to A), whereas carbon substrates had a moderate effect (treatments B to D compared to F). Increasing the amount of NZVI in the solution (treatment D compared to B) resulted in a more effective and faster nitrate reduction. In addition, comparing treatment E with C, it can be observed that a Maize cobs to beech sawdust ratio of 1:1 was more effective than a ratio of 2:1.

The results indicate that the presence of the carbon substrates lead to a higher ammonium production over time. In all tests, the ammonium concentration increased over time up to a maximum value, which depended on the quantity of NZVI used in the solution. In a second stage, a constant value or a decrease in the ammonium concentration was observed. Treatment F (no carbon substrates) corresponded to the lowest production of ammonium (8.5 mg/l at the seventh day), while a slightly higher production was observed for treatment A (20 mg/l). For treatments A to E, the decrease in the ammonium concentration after the peak value can be associated to the increasing pH, which is a favorable condition for ammonium stripping. Increasing the concentration of NZVI in the treatment D delayed the time of the peak ammonium concentration of one day.



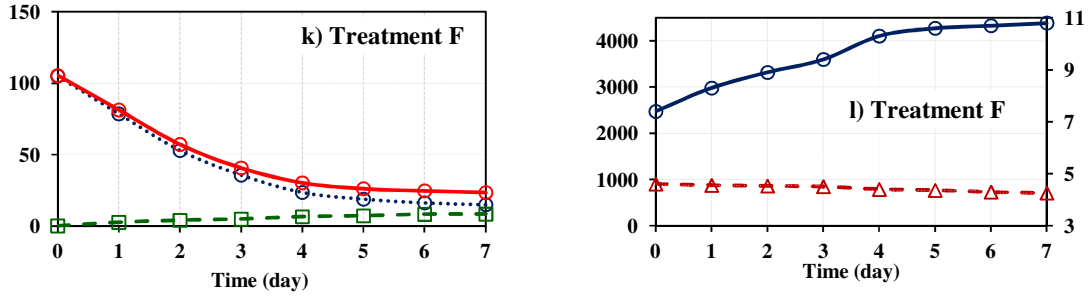


Figure 4: Temporal variation of nitrate, ammonium, total nitrogen (TN), pH, and EC in solution of bottles for different treatments.

5.2.1 Denitrification Kinetics

The processes of nitrate degradation and ammonium production and stripping can be described by a simple pseudo first-order kinetic model (Choe et al., 2004; Hwang et al., 2011):

$$\frac{dC_{NO_3}}{dt} = -k_1 C_{NO_3} \xrightarrow{\text{Integrating}} C_{NO_3}^t = C_{NO_3}^0 e^{-k_1 t} \quad (4)$$

$$\begin{aligned} \frac{dC_{NH_4}}{dt} = k_1(C_{NO_3}^0 - C_{NO_3}) - k_2 C_{NH_4} \xrightarrow{\text{Integrating}} C_{NH_4}^t = & \left(\frac{C_{NH_4}^0}{C_{NO_3}^0} + \frac{k_1^2}{k_2^2 - k_1 k_2} \right) C_{NO_3}^0 e^{-k_2 t} \\ & + \left(\frac{k_1}{k_1 - k_2} \right) C_{NO_3}^0 e^{-k_1 t} + \frac{k_1}{k_2} C_{NO_3}^0 \end{aligned} \quad (5)$$

where $C_{NO_3}^t$ and $C_{NH_4}^t$ are the aqueous nitrate and ammonium concentrations at time t , respectively, $C_{NO_3}^0$ and $C_{NH_4}^0$ are the initial nitrate and ammonium concentration, respectively, k_1 and k_2 are the nitrate reduction ammonia stripping constant, respectively.

The kinetic constants k_1 and k_2 were optimized by fitting the experimental data of nitrate and ammonium concentrations in batch tests to the Equations (4) and (5) (Table 3). The results indicate that the pseudo first-order kinetic model can correctly simulate the process for all treatments (except the ammonium stripping constant for treatment F, see the corresponding R^2 values). The value of fitted kinetic constant k_1 is directly proportional to the NZVI dosage in the solution, since the maximum and minimum value of k_1 were obtained for treatments D and A respectively (Table 3). The minimum kinetic stripping constant k_2 (0.071 day^{-1}) was obtained for treatment A (no NZVI), while the maximum k_2 (2.28 day^{-1}) was obtained for treatment F (no carbon substrate). The k_2 value for treatment F is significantly higher compared to the other

treatments (four times the value obtained for treatment D and thirty times the value for treatment A). The value of $R^2 = 0.782$ obtained for treatment F confirmed that the assumption of pseudo first-order kinetics is not accurate when NZVI alone is used. This finding is in agreement with the results obtained by [Hwang et al. \(2011\)](#).

Table 3: Estimated constants of nitrate reduction, ammonium production and stripping for six treatments.

Treatment	Nitrate Reduction Constants		Ammonium Stripping Constants	
	k_1 (day ⁻¹)	R^2	k_2 (day ⁻¹)	R^2
A	1.05	0.985	0.07	0.998
B	1.73	0.890	0.35	0.965
C	1.95	0.931	0.29	0.970
D	2.61	0.973	0.52	0.821
E	1.86	0.945	0.35	0.928
F	0.29	0.963	2.29	0.782

5.2.2 Gas Production

The variation over time of total nitrogen (TN) in solution provides information on the amount of gasses produced by the denitrification reactions. In treatment A only 27% of initial TN (TN₀) in the solution was converted to gas (N₂ and or NH₃) at the end of experiment, while 78% of TN₀ was reduced in treatment F. For the other treatments (B to E), the percentage of the TN remaining in solution at the end of the test was in the range 50-65%. This can be due to a higher rate of chemical denitrification in water in comparison to biological or bio-chemical denitrification. In addition, nitrate was converted to ammonium ion followed by ammonia stripping under an alkaline condition (up to pH=10), which lead to a decrease in the total amount of nitrogen in solution.

5.2.3. pH variation

The temporal evolution of the solution pH for the six treatments from the initial value of 7.4±0 is also shown in Figure 4. According to these results, three distinct pH variation trends can be observed:

- a) The pH value decreased from 7.4 to 3.1 at the end of the experiment in treatment A (no NZVI). This may be due to the presence of carbon dioxide and other acids, which are produced during the metabolic process of the bacteria (Hsu et al., 2011):
- b) The pH increased up to 10.8 for treatment F (only NZVI). This can be attributed to the production of hydroxide ions according to Equations (1) and (2). As the pH increases, the rate of NZVI reaction decreases due to the formation of the oxide layer on the surface of the particles. Furthermore, a high pH lead to an unfavorable environment (e.g. accumulation of ammonium ions) for microbial reduction. The increased pH provided thermodynamically favorable conditions for the production of nitrogen gas and stripping of ammonium as a final product with a consequent significant decrease of the TN in the solution.
- c) A minor decrease in pH (between 0.6 to 1.1 with respect to the initial value) after one day, followed by a moderate increase (0.3-1.4) up to the end of the experiment was observed for treatments B to D. The conflicting effects of NZVI and denitrifier bacteria seem to have caused these variations, since NZVI tends to increase pH (due to hydroxide ion production) and bacterial activity tends to decrease the pH (due to the production of carbonic acid). On the other hand, when the acidity of the solution increases, the surface of the particles is refreshed. As a result, the rate of ammonium production by the nanoparticles increases and reaches a peak value. Subsequently, the rate of ammonium production slightly decreases, probably due to accumulation of some toxic ions (e.g. ammonium) in the solution, preventing further microbial growth.

The intermediate pH observed in tests B to D, together with a good degree of removal of nitrate, suggests that both abiotic (by NZVI) and biotic degradation were occurring in parallel, with opposite effects on the pH. The limitation of changes in pH can help increasing the degradation rates of both processes, and contribute to shift the equilibrium of the degradation reactions toward the products.

5.2.3. Electrical Conductivity

The electrical conductivity (EC) trend for treatment F (containing only the NZVI particles) exhibited a linear decrease during the experiment, from the initial value of 903 $\mu\text{S}/\text{cm}$ to 710 $\mu\text{S}/\text{cm}$ on the seventh day (21% of EC reduction). This is due to the capability of the NZVI particles to reduce a wide range of cations and anions with different valences in the natural

groundwater. Conversely, the EC increased exponentially when the carbon substrates were used individually (treatment A) or mixed with the NZVI (treatments of B to E). The rate of EC growth ranged from 200% (for treatment A) to 380% (for treatment D). This may be due to the increased pH value for higher production of cations and anions in the solution by the carbon substrates. The increase of NZVI dosage (treatment D compared to treatments B and C) resulted in the increase of EC. Based on the results on the batch tests, the mixture of maize cobs, beech sawdust and NZVI in the weight ratio of 10:5:1 (treatment D) was identified as the most efficient in nitrate degradation, and was used as reactive material in the bench-scale aquifer test.

5.3. Bench-Scale Aquifer

5.3.1 Numerical Flow Simulations

The configuration of the bench-scale NPRWs array (i.e the most efficient number of wells N_W , L_W and D_W), was designed based on a numerical flow and particle tracking model developed using MODFLOW and MODPATH. Several simulations were run, corresponding to different candidate configurations of the array, and their performances were compared to find the optimum in terms of efficacy of the wells in capturing the flow pathlines. Mass balance errors lower than 0.05% were obtained for all simulation runs. The optimum values of N_W , L_W and D_W were obtained equal to 4, 65 mm, and 35 mm, respectively. In other words, one row composed by four wells with diameter 35 mm filled by reactive materials (NZVI mixed by carbon substrates) with uniform spacing of 65 mm were placed in the aquifer bench-scale model, perpendicular to the flow direction, as shown in Figure 3.

An example of simulation results in terms of capture zone of NPRWs and flow pathlines around the wells, simulated by PMPATH, is shown in Figure 5. A capture zone of each well wide as twice the well diameter ($\sim 1.8 D_W$) was obtained for a contrast between the average hydraulic conductivity of the aquifer (2.10 m/d) and of the reactive materials (treatment D, $K_{RM} = 34.60$ m/d) with equal to $K_{RM}/\bar{K} = 16.50$.

5.7. Denitrification in the Bench-Scale Aquifer Model

The nitrate concentration in all upstream and downstream piezometers (P_1 to P_7) every 24 hours (corresponding to the residence time in the aquifer model) as shown in Figure 6. Since the monitored nitrate concentrations in the upstream piezometers (P_1 and P_2) are very similar in all

circulations, they are reported here as an average (U.S.) in Figure 6. Nitrate concentration below acceptance criteria was found in most of the down-gradient monitoring piezometers of the NPRWs system (P3 to P7) after 13 days (approximately 13 circulations). This suggests that similar results (95% nitrate removal) could be obtained if 13 arrays of NPRWs are considered along the flow direction. The trend of nitrate reduction monitored in downstream piezometers (P3 to P7) showed moderate peaks, which can be due to by-passing of some un-treated streamline of polluted groundwater between wells without entering in contact with the reactive materials. This effect would be mitigated at a large scale if the consecutive arrays of NPRWs are non-aligned.

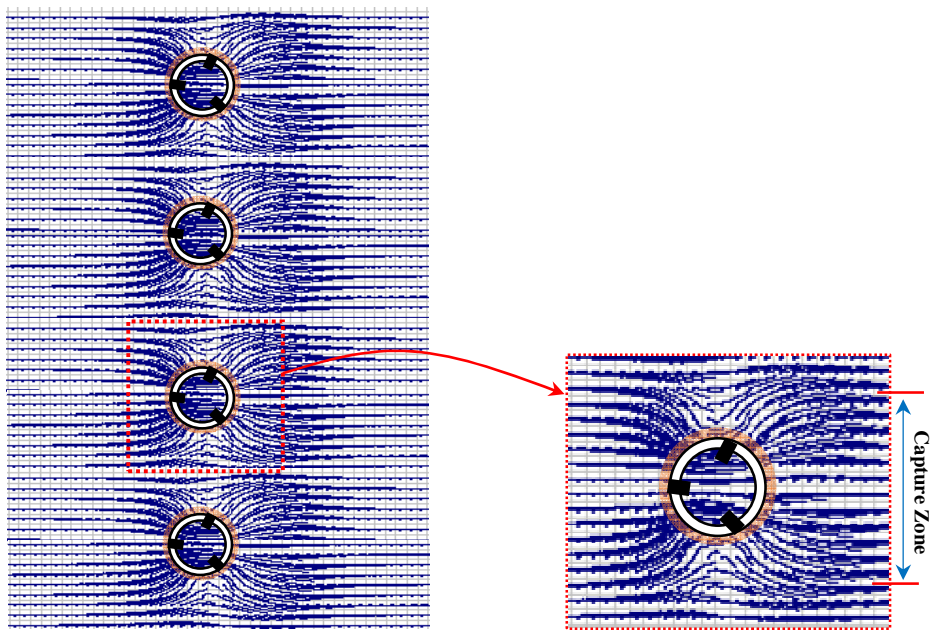


Figure 5: Steady state capture area for the wells in the first layer by tracking 200 particles forward from the upstream boundaries.

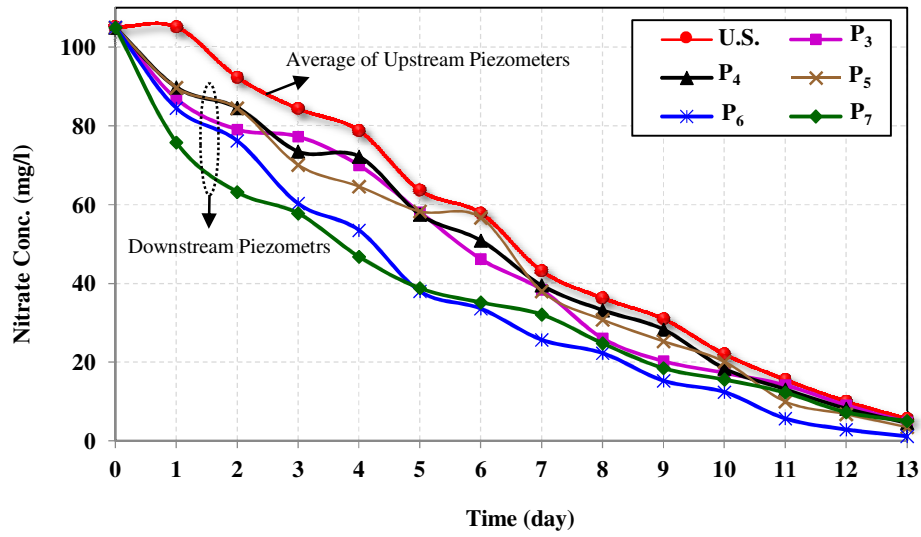


Figure 6: Rate of nitrate reduction from groundwater by NPRWs system at monitoring piezometers. The abbreviations refer to samples collected in piezometers downstream ("P3" to "P7") and upstream ("U.S.") the wells system.

For a deeper investigation of the nitrate reduction process in the NPRWs system, statistics of nitrate reduction percentage in each circulation (Cir1 to Cir13) in terms of minimum, maximum, mean, and upper and lower quintiles in the downstream piezometers (P3 to P7) are shown in a boxplot in Figure 7. The values were computed for each circulation with respect to the inlet nitrate concentration at the corresponding circulation. It is noticeable that the maximum nitrate reduction is occurring during the fourth to sixth circulation (Cir4 to Cir6), whereas the mean nitrate reduction declines when circulation of plume increases.

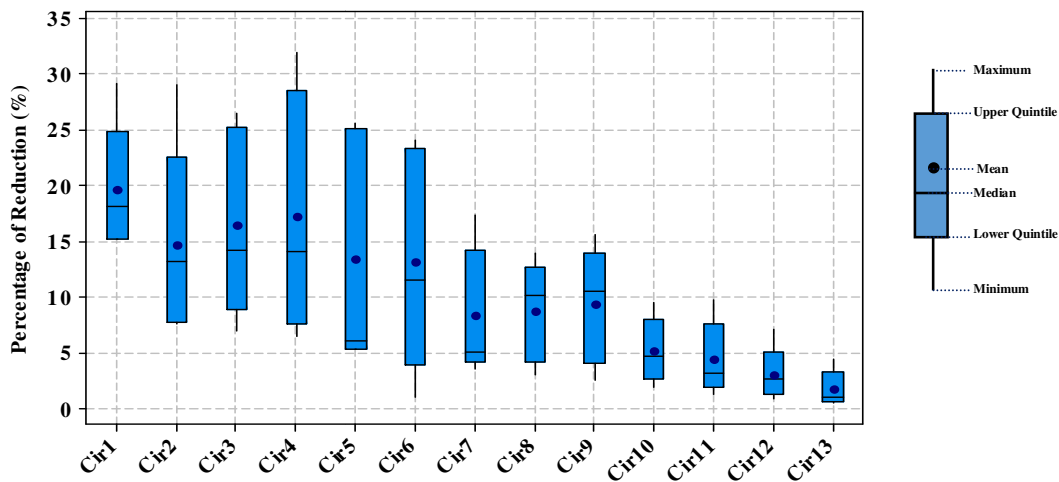


Figure 7: Statistics of nitrate reduction in the downstream piezometers of the bench-scale aquifer after each circulation.

The average cumulative percentage of nitrate removal observed in the downstream piezometers at each circulation indicates that the degradation half-life of nitrate in the NPRWs system is approximately six days, and more than 90% of NO_3^- was degraded after about 11 days.

The ammonium production was also monitored in the upstream (P1 and P2) and downstream piezometers (P3 to P7) (Figure 8a). No significant increase in NH_4^+ concentration was observed during the first two days. From the third day, the rate of NH_4^+ production started increasing, up to 0.51-0.55 mg/l in the 13th day. It should be mentioned that the final value is very close to the maximum allowable level in drinking water (0.5 mg/l).

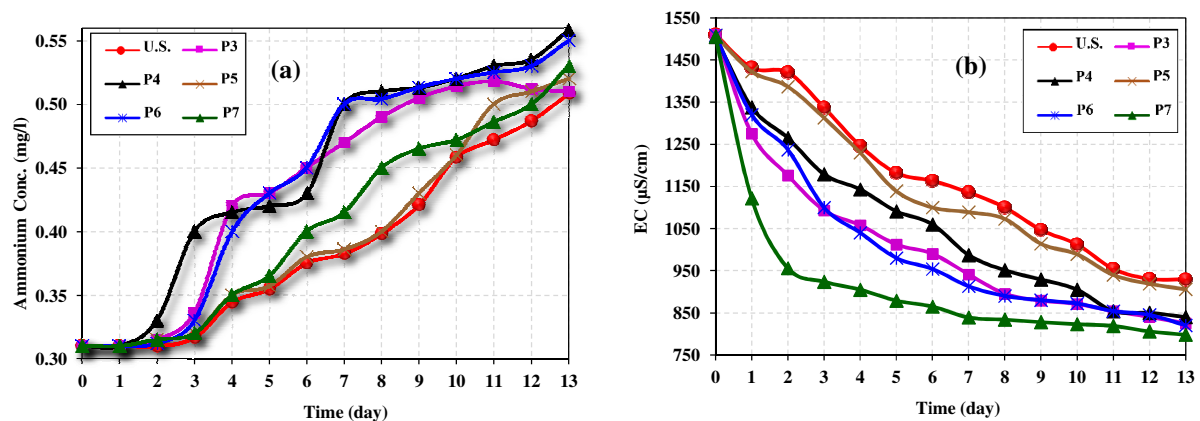


Figure 8: Ammonium concentration (a) and EC (b) in upstream and downstream piezometers of the NPRWs system during the experiment time. The initial values of ammonium concentration and EC were, respectively, ~ 0.3 mg/l and $1506 \pm 8 \mu S/cm$.

Finally, the trend of groundwater electrical conductivity (EC) observed in the upstream (P1 and P2) and downstream (P3 to P7) piezometers is reported in Figure 8b. The EC downstream the NPRWs system decreased from $1506 \pm 8 \mu S/cm$ to 798-929 $\mu S/cm$ during the 13 days, which denotes an EC attenuation from 38% to 47%. This can be attributed to the capability of the NZVI particles to reduce the ions with different valence which are frequently included in the natural groundwater (e.g., Na^+ , Mg^{2+} , Ca^{2+} , CO_3^{2-} , Cl^-), in spite of generation of the NH_4^+ and OH^- (Ruangchainikom et al., 2006).

It is finally worth to mention that the average pH of the groundwater downstream the NPRW array did not show significant variations compared to the upstream ones. Only a slight increase of pH averagely equal to 0.2 was observed. Additional experiments were also performed to investigate the effects of the eventual formation of precipitates in the reactive zone on the hydraulic properties of the bench-scale aquifer (data not reported). The results indicated an insignificant reduction of the hydraulic conductivity (~3%) after 13 days.

6. Conclusions

The efficacy of Non-Pumping Reactive Wells (NPRWs) for the biochemical remediation of nitrate-contaminated groundwater was assessed through laboratory bench-scale studies, which represent the first step of a full-scale remediation of a deep contaminated aquifer in Iran. The reactive material to be emplaced in the wells was first selected based on batch degradation tests using several combinations of carbon substrates (Maize cobs and beech sawdust) and nanoscale zero-valent iron, alone or in combination with the others. The mixture of maize cobs, beech sawdust and NZVI in the weight ratio of 10:5:1 showed the best performance, in particular compared to the application of the individual materials, and was applied for the bench-scale test for the denitrification under flow conditions. The two carbon substrates support and promote the growth of denitrifying bacterial communities, which in turn take advantage of the abiotic chemical reactions occurring at the NZVI surface (namely, production of hydrogen gas and hydroxide anions). The NZVI also contribute to the reduction of nitrate, and the reaction kinetics is enhanced by the nearly neutral pH established by the concurring bacterial activity.

Since this study is focused on the final application of the system at the full scale, the water from the contaminated aquifer was directly employed in the laboratory tests. The authors are aware that this practical approach makes more complicated the full comprehension of the reaction mechanisms occurring in the batch and the bench scale model, but provides more reliable results for the up-scaling of the technology to the field. In this sense, further studies using synthetic contaminated water can be envisioned in the future for a deeper and more quantitative insight in the reaction mechanisms. It is worth to mention that the use or recirculation in the bench-scale model introduces a certain degree of homogenization, and consequently the inlet concentration observed in the laboratory is not the same which would enter the NPRWs in the second to 13th row in the field. The use of non-aligned arrays of NPRWs would mitigate this difference.

However, a pilot installation at the field will be necessary before the realization of the full-scale intervention.

The NPRWs behave as a discontinued PRB, and consequently multiple staggered arrays of wells are to be drilled. The bench-scale model includes a single array of NPRWs, but it was used to reproduce the denitrification performance of several arrays by re-circulating the outflow at the inlet. Since each re-circulation mimics the flow of the contaminated water through a single array, this approach was used for the determination of the number of sequential arrays in the full scale application. The results indicated that the nitrate present in the groundwater at the concentration of 105 mg/l of NO_3^- was completely degraded in most of the monitoring piezometers down-gradient of the NPRWs system after 13 days, corresponding to the passage through 13 parallel arrays of NPRWs.

Beside the reactivity of the NPRWs filling material, the spacing among wells of the same array and between subsequent arrays, as well as the well diameter, are crucial parameters in the design of the full-scale intervention. In this work the design of the bench-scale model, represented by a single array of wells, was carried out using the numerical model PMPATH, which can be applied also for the full-scale design. The NPRWs were preferred for this application to the more classical configuration of a continuous PRB due to the specific application envisioned for the system: the contamination is located at great depth, with the water table at approximately 36 m below the ground level, and the bedrock at 100 m b.g.l. or lower. Such depth makes the option of an excavated PRB unfeasible. Moreover, concentrating the NZVI in the NPRWs system may reduce the risk of particle release in the environment over the long-time frame, even if the mobility of bare (non-stabilized) NZVI is very limited under natural flow conditions (Tosco et al., 2014).

NPRWs also have the advantage of an easier restoration of the reactive material in comparison with common PRB configurations. This aspect is crucial for the specific application envisioned here, since in this case the removal of the (diffuse and multiple) sources is not feasible: the high importance of the water resource in the region, and consequently the risk associated to its contamination, require the full scale system to be installed in a short time, and be regenerated quite often. Furthermore, NPRWs can be more easily adapted to variations in concentration and flow direction over time, compared to PRBs: in case of an increase in the inlet concentration, further arrays of wells can be drilled, or existing ones can be discontinued if a significant

decrease in the inlet concentration is observed. The arrays can also be extended laterally in case of observed bypass of the reactive wells front. This is particularly useful in the full scale application envisioned here, since the nitrate contamination is caused by several sources, not all characterized into details, and changes in the nitrate concentration and plume width cannot be excluded for the future. Finally, it is worth to mention that, even though the realization of multiple arrays of reactive wells, as well as the use of NZVI, will imply a high cost for the intervention, it is justified due to the importance of the water resource, which is the only significant fresh water source in the area.

7. Acknowledgments

This research was made by funding from Research Committee of Chahar-Mahal and Bakhtiari Regional Water Organization (Iran) under contact No. 310/91/1537.

8. References

- An, Y., Li, T., Jin, Z., Dong, M., and Li, Q. (2010). Nitrate degradation and kinetic analysis of the denitrification system composed of iron nanoparticles and hydrogenotrophic bacteria. *Desalination*, 252(1-3): 71-74.
- Boley, A., W.R. Müller, and G. Haider. (2002). Biodegradable polymers as solid substrate and biofilm carrier for denitrification in recirculated aquaculture systems. *Aquaculture Engineering* 22: 75–85.
- Bremner, J.M., Shaw, K. (1958). Denitrification in soil: II. Factors affecting denitrification. *J. Agr. Sci.* 51: 40-52.
- Cameron, S.C., Schipper, L.A. (2010). Nitrate removal and hydraulic performance of carbon substrates for potential use in denitrification beds. *Ecol. Eng.* 36: 1588-1595.
- Cheng, I.F., Muftikian R., Fernando, Q., and Korte, N. (1997). Reduction of nitrate to ammonia by zero-valent iron. *Chemosphere* 35(11): 2689-2695.

- Chiang, W.H., Kinzelbach, W. and Rausch, R. (1998). *Aquifer Simulation Model for Windows - Groundwater flow and transport modeling, an integrated program*. Gebriider Bomtraeger, Berlin Stuttgart. ISBN 3-443-01039-3.
- Choe S.H., Ljestrland H.M., Khim J. (2004). Nitrate reduction by zero-valent iron under different pH regimes, *Appl. Geochem.* 19: 335–342.
- Comba, S., Martin, M., Marchisio, D., Sethi, R., Barberis, E. (2011a). Reduction of nitrate and ammonium adsorption using microscale iron particles and zeolite. *Water, Air, and Soil Pollution*, 223(3): 1079-1089.
- Comba, S., Molfetta, A.D., and Sethi R. (2011b). A Comparison Between Field Applications of Nano-, Micro-, and Millimetric Zero-Valent Iron for the Remediation of Contaminated Aquifers, *Water Air Soil Pollut*, 215:595–607. DOI 10.1007/s11270-010-0502-1.
- Cook, S. (2009). Assessing the use and application of zero-valent iron nanoparticle technology for remediation at contaminated sites, Prepared for the US Environmental Protection Agency. [Online] Available at: <http://www.clu-in.org/download/studentpapers/Zero-Valent-Iron-Cook.pdf>
- Day, S.R., S.F. O'Hannesin, and L. Marsden. (1999). Geotechnical techniques for the construction of reactive barriers. *Journal of Hazardous Materials*, 67: 285–297.
- Dejournett, T. and Alvarez, P.J.J. (2000). Combined microbial-Fe(0) system to remove nitrate from contaminated groundwater. *Bioremediation Journal*, 4:149-154.
- Della Rocca, C., Belgiorno, V., and Meriç, S. (2007a). Overview of in-situ applicable nitrate removal processes. *Desalination*, 204: 46–62.
- Della Rocca, C., V. Belgiorno, and S. Meriç. (2007b). Heterotrophic/autotrophic denitrification (HAD) of drinking water: prospective use for permeable reactive barrier. *Desalination*, 210: 194-204.
- Delwiche, C.C. (1981). *The Nitrogen Cycle and Nitrous Oxide in Denitrification, Nitrification and Atmospheric Nitrous Oxide*. New York: Wiley Interscience.
- Freethey, G.W., Naftz, D.L., Rowland, R.C., and Davis, J.A. (2002). Deep aquifer remediation tools: Theory, design, and performance modeling. In: Naftz DL, Morrison SJ, Davis JA, Fuller CC, eds. *Handbook of groundwater remediation using permeable reactive barriers*. Amsterdam: Academic Press, 133-61.

- Garcia-Armesto, M.R., Prieto, M.L., Garcia-Lopez, Otero A., Moreno B. (1993). Modern microbiological methods for foods: colony counting and direct count methods. A review, *Microbiologia*, 9 (1): 1–13.
- Gastone F., Tosco T., Sethi R. (2014). Green stabilization of microscale iron particles using guar gum: bulk rheology, sedimentation rate and enzymatic degradation. *Journal of Colloid and Interface Science* 421, 33-43.
- Gayle, B.P., Boordman, G.D., Serrard, J.H., and Benait, R.E. (1989). Biological denitrification of water. *Environ. Eng. Div.*, 115: 930-935.
- Gillham, R.W., and O'Haneisin S.F. (1994). Enhanced degradation of halogenated aliphatic by zero-valent iron. *Ground Water*, 32: 958–967.
- Greenan, C.M., Moorman, T.B., Kaspar, T.C., Parkin, T.B., and Jaynes, D.B. (2006). Comparing carbon substrates for denitrification of subsurface drainage water. *J Environ Qual.*, 35: 824–829.
- Gu, B., D.B. Watson, D.H. Phillips, and L. Liang. (2002). Biochemical, Mineralogical, and Hydrological Characteristics of an Iron Reactive Barrier used for Treatment of Uranium and Nitrate., 305–342. San Diego: Academic Press.
- Hosseini, S. M., Ataie-Ashtiani, B., and Kholghi, M. (2011). Nitrate reduction by nano-Fe/Cu particles in packed column, *Desalination*, 276: 214–221/<http://www.nanoiron.cz/en/nanofer-star>.
- Huang, C.P., Wang, H.W., and Chiu, P.C. (1998). Nitrate reduction by metallic iron. *Water Research*, 32:2257–2264.
- Huang, P., Ye, Z., Xie, W., Chen, Q., LI, J., Xu, Z., and Yao, M. (2013). Rapid Magnetic Removal of Aqueous Heavy Metals and their Relevant Mechanisms Using Nanoscale Zero Valent Iron (nZVI) Particles. *Water research*, 47, 12, 4050-4058.
- Huang, W.E., Oswald, S.E., Lerner, D.N., Smith, C.C., and Zheng, C. (2003). Dissolved Oxygen Imaging in a Porous Medium to Investigate Biodegradation in a Plume with Limited Electron Acceptor Supply, *Environmental Science & Technology*, 37: 1905-1911.
- Huang, Y.H., and Zhang, T.C. (2004). Effects of low pH on nitrate reduction by iron powder. *Water Research*, 38: 2631–2642.
- Hudak, P.F. (2008). Evaluation of reactive well networks for remediating heterogeneous aquifers. *J Environ Sci Health*, 43 (7): 731-737.

- Karn, B., Kuiken, T. and Otto, M. (2009). Nanotechnology and in situ remediation: A review of the benefits and potential risks, *Environmental Health Perspectives*, 117, 12, 1823-1831. DOI:10.1289/ehp.0900793.
- Keller, A.A., Garner, K., Miller, R.J., and Lenihan, H.S. (2012). Toxicity of nano-zero valent iron to freshwater and marine organisms, *PloS one*, 7,8, e43983.
- Li, L., M. Fan, R.C. Brown, J.V. Leeuwen, J. Wang, W. Wang, Y. Song, and P. Zhang. (2006). Synthesis, properties and environmental applications of nanoscale iron-based materials: A review. *Environmental Science and Technology*, 36 (5): 405–431.
- Li, N., Jin, Z.H., Li, T.L., Mei-ying, D., Hong-cai, X., Zhao-hui, J. (2011). Effect of bio film on nanoscale zero-valent iron-microorganism removing NO₃-N in groundwater, *Environ. Sci.*, 32(6): 1620–1626.
- Lien, H.L., and Wilkin, R.T. (2005). High-level arsenite removal from groundwater by zero-valent iron, *Chemosphere*, 59(3): 377-386.
- Liu, F., Huang G., Fallowfield H., Guan H., Zhu L., and Hu H. (2014). Study on Heterotrophic-Autotrophic Denitrification Permeable Reactive Barriers (HAD PRBs) for In Situ Groundwater Remediation. Springer Berlin Heidelberg, ISBN: 978-3-642-38153-9.
- Luna M., Gastone F., Tosco T., Sethi R., Velimirovic M., Gemoets J., Muyschond R., Sapion H., Klaas N., and Bastiaens L. (2015). Pressure-controlled injection of guar gum stabilized microscale zerovalent iron for groundwater remediation. *Journal of contaminant Hydrology*, DOI: 10.1016/j.jconhyd.2015.04.007
- McDonald, M.G., and Harbaugh, A.W., 1988, A modular three-dimensional finite-difference ground-water flow model: Techniques of Water-Resources Investigations of the United States Geological Survey, Book 6, Chapter A1, 586 p.
- Naftz, D.L., S.J. Morrison, J.A. Davis, and C.C. Fuller. (2002). Editors, *Handbook of Groundwater Remediation Using Permeable Reactive Barriers: Applications to Radionuclides, Trace Metals, and Nutrients.*, San Diego, CA: Academic Press.
- Novak, P., Daniels, L., and Parkin, G. (1998). Enhanced dechlorination of carbon tetrachloride and chloroform in the presence of elemental iron and *Methanosarcina barkeri*, *Methanosarcina thermophila*, or *Methanosaeta concillii*. *Environ. Sci. Technol.* 32: 1438-1443.

- Nurmi, J.T., and Tratnyek P.G. (2008). Electrochemical studies of a packed iron powder electrode in solutions containing carbonate and natural organic matter. *Journal of Corrosion Science* 50: 144.
- O'Carroll, D., Sleep, B., Krol, M., Boparal, H., and Kocur, C. (2013). Nanoscale zero valent iron and bimetallic particles for contaminated site remediation, *Advances in Water Resources*, 51, 104-122.
- Obiri-Nyarko, F., Grajales-Mesa, S.J., Malina, G. (2014). An overview of permeable reactive barriers for in situ sustainable groundwater remediation, *Chemosphere*, 111: 243-259.
- Pearson, F.H., and McDonnell, A.J. (1975). Use of crushed limestone to neutralize acid wastes. *Journal of the Environmental Engineering Division*, 101(1): 139-158.
- Pherat, T., Long, T.C. Lowry, G.V. and Veronesi, B. (2009). Partial oxidation (“aging”) and surface modification decrease the toxicity of nanosized zerovalent iron, *Environmental Science and Technology*, 43, 1, 195-200.
- Philips, D.H., Gu, B., Watson, D.B., Roh, Y., Liang, L., and Lee, S.Y. (2000). Performance evaluation of a zerovalent iron reactive barrier: mineralogical characteristics. *Environmental Science & Technology*, 34: 4169–4176.
- Pollock, D. W. (1994). User's Guide for MODPATH/MODPATH-PLOT, Version 3: A particle tracking post-processing package for MOD- FLOW, the U.S. Geological Survey finite-difference ground- water flow model, US Geological Survey Open-File Report 94- 464, 249 p.
- Pollock, D.W. (1994). User's Guide for MODPATH/MODPATH-PLOT, Version 3: A particle tracking post-processing package for MODFLOW, the U. S. Geological Survey finite-difference ground-water flow model, U. S. GEOLOGICAL SURVEY, Report 94-464.
- Renn, O. and Rocco, M. (2006). Nanotechnology Risk Governance, White Paper No. 2. [Online] Available at: http://www.irgc.org/IMG/pdf/IRGC_white_paper_2_PDF_final_version-2.pdf
- Robertson, W.D., and Cherry, J.A. (1995). In Situ Denitrification of Septic-System Nitrate Using Reactive Porous Media Barriers: Field Trials. *Ground Water*, 33(1): 99-111.
- Royal Society (2005). Report of workshop on potential health, environmental, and societal impacts of nanotechnologies. London, 25 November 2005.

- Ruangchainikom, C., Liao, C.H., Anotai, J., and Lee, M.T. (2006). Characteristics of nitrate reduction by zero-valent iron powder in the recirculated and CO₂-bubbled system. *Water Research*, 40: 195–204.
- Schafer, W. and Kinzelbach, W. (1992). Stochastic modeling of in situ bioremediation in heterogeneous aquifers. *Journal of Contaminant Hydrology*, 10: 47-73.
- Schipper, L.A., and Vojvodić-Vuković, M. (2000). Nitrate removal from groundwater and denitrification rates in a porous treatment wall amended with sawdust. *Ecological Engineering* 12: 269–278.
- Shao, L., Xu, Z.X., Jin W., and Yin H.L. (2009). Rice Husk as Carbon Source and Biofilm Carrier for Water Denitrification, *Polish J. of Environ. Stud.* 18(4): 693-699.
- Shin, K.H., and Cha, D. (2008). Microbial reduction of nitrate in the presence of nanoscale zero-valent iron, *Chemosphere*, 72: 257-62.
- Su, C. Puls, R.W., Krug, T.A., Watling, M.T., O'hara, S.K., Quinn, J.W. and Ruiz, N.E. (2012). A two and half-year-performance evaluation of a field test on treatment of source zone tetrachloroethene and its chlorinated daughter products using emulsified zero valent iron nanoparticles, *Water Research*, 46, 5071-5084.
- Su, C., and Puls, R.W. (2004). Nitrate Reduction by Zerovalent Iron: Effects of Formate, Oxalate, Citrate, Chloride, Sulfate, Borate, and Phosphate. *Environmental Science & Technology*, 38(9): 2715-2720.
- Taylor, S.W., and Jaffé P.R. (1991). Enhanced In-Situ Biodegradation and Aquifer Permeability Reduction, *J. of Environmental Engineering, ASCE*, 117(1): 25-46.
- Till, B., Weathers, L.G., and Alvarez, P.J.J. (1998). Fe(0)-Supported Autotrophic Denitrification, *Environ. Sci. Technol.* 32: 634-639.
- Till, B.A., Weathers, L.J., and Alvarez, P.J.J. (1998). Fe(0)-supported autotrophic denitrification. *Environ. Sci. Technol.* 32(5): 634-639.
- Tosco, T., Petrangeli Papini, M., Cruz Viggi, C. Sethi, R. (2014). Nanoscale iron particles for groundwater remediation: a review. *Journal of Cleaner Production*, 77, 10-21.
- Tratnyek, P.G. and Johnson, R.L. (2006). Nanotechnologies for environmental cleanup, *Nano Today*, 1, 2, 44-48.
- Tratnyek, P.G., Scherer, M.M., Johnson, T.J., and Matheson, L.J. (2003). Permeable reactive barriers of iron and other zero-valent metals, in: M.A. Tarr (Ed.), *Chemical Degradation*

- Methods for Wastes and Pollutants: Environmental and Industrial Applications, Marcel Dekker, New York, pp. 371–421.
- USEPA. (1998). Field Application of in situ remediation technologies: ground-water circulation wells. US Environmental Protection Agency. Publication # EPA 542-R-98-009, Washington, DC.
- Van Nooten, T., Diels L., and Bastiaens L. (2008). Design of a multifunctional permeable reactive barrier for the treatment of landfill leachate contamination: Laboratory column evaluation. *Environmental Science and Technology*, 42(23): 8890-8895.
- Velimirovic, M.; Tosco, T.; Uyttebroek, M.; Luna, M.; Gastone, F.; De Boer, C.; Klaas, N.; Sapon, H.; Eisenmann, H.; Larsson, P.-O.; Braun, J.; Sethi, R., Bastiaens, L. (2014). Field assessment of guar gum stabilized microscale zerovalent iron particles for in-situ remediation of 1,1,1-trichloroethane. *Journal of Contaminant Hydrology* 2014, 164(0), 88-99.
- Volokita, M., Abehovich, A., and Soares, M.I.M. (1996). Denitrification of groundwater using cotton as energy source. *Water Science and Technology*, 34(1–2): 379-385.
- Warneke, S., Schipper, L.A., Matiassek, M.G., Scow, K.M., Cameron, S., Bruesewitz, D.A., and McDonald, I.R. (2011). Nitrate removal, communities of denitrifiers and adverse effects in different carbon substrates for use in denitrification beds, *Water Research*, 45: 5463-5475.
- Weathers, L.J., Parkin, G.F., and Alvarez, P.J.J. (1997). Utilization of Cathodic Hydrogen as Electron Donor for Chloroform Cometabolism by a Mixed Methanogenic Culture. *Environ. Sci. Technol.*, 31(3): 880-885.
- Westerhoff, P., and James, J. (2003). Nitrate removal in zero-valent iron packed columns. *Water Research*, 37(8): 1818-1830.
- WHO. (2005). *Guidelines for Drinking Water Quality*, 3rd ed, Geneva: World Health Organization.
- Wilson, R.D., Mackay, D.M., and Cherry, J.A. (1997). Arrays of unpumped wells for plume migration control by semi-passive in situ remediation, *Ground Water Monitoring and Remediation*, 17: 185-193.
- Wilson, R.D., Mackay, D.M., and Cherry, J.A. (1997). Arrays of unpumped wells for plume migration control by semi-passive in situ remediation, *Ground Water Monitoring and Remediation*, 17: 185-193.

Zhang, W.X. (2003). Nanoscale iron particles for environmental remediation: an overview, J. Nanoparticle Res., 5: 323-332.